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(54) Title: N6-SUBSTITUTED 9-METHYLADENINES: A NEW CLASS OF ADENOSINE RECEPTOR ANTAGONISTS

(57) Abstract

A series of N6-substituted adenines are disclosed to be antagonists of A_2-adenosine receptor-mediated stimulation of adenylate cyclase in A_2-adenosine receptors and antagonists of A_1-adenosine receptor-mediated inhibition of adenylate cyclase. These compounds are useful in reversal of adenosine-mediated lipolysis, reversal of aenosine-mediated deleterious cardiovascular effects (conduction defects, hypotension), reversal of adenosine-mediated vascular actions in kidney, bronchodilation, antiarrhythmic action, reversal of adeno-mediated relaxation of smooth muscle, anti-narcoleptic action, CNS stimulation, and blockade of adenosine mediated inhibition of neurotransmitter release.

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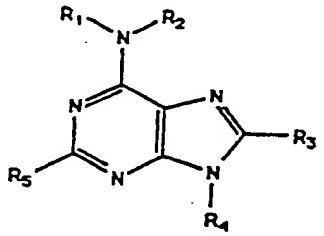
N^6-SUBSTITUTED 9-METHYLADENINES: A NEW CLASS OF ADENOSINE RECEPTOR ANTAGONISTS

SUMMARY OF THE INVENTION

Novel compounds and a method of using them to antagonize adenosine receptors are provided wherein the compounds are represented by the general formula:



wherein R_2 is selected from the group consisting of cycloalkyl radicals having from 3 to 8, preferably 3 to 7, ring carbon atoms, alkyl radicals having from 1 to 10 carbon atoms, aryl radicals having from 6 to 13, preferably 6 to 10, carbon atoms, aralkyl radicals having from 7 to 14, preferably 7 to 10, carbon atoms, and heteroatom- and halogen-substituted derivatives thereof wherein said heteroatom may be selected from the group consisting of nitrogen, phosphorus, sulfur and oxygen; R_1 may be hydrogen or R_2, and R_3 is selected from the group consisting of hydrogen, halogen, amine, carboxy, thio, sufonate, sulfonamide, sulfone, sulfoxamide, phenyl, alkyl-substituted amine, cycloalkyl-substituted amine, alkyl radicals having from 1 to 10 carbon atoms, and cycloalkyl radicals having from 3 to 8, preferably 5 to 6, ring carbon atoms. R_4 is selected from the group consisting of benzyl, phenyl, and

alkyl groups comprising from 1 to 4 carbon atoms, wherein said alkyl group can be substituted with oxygen, for example ethers and alcohols. R_5 is selected from the group consisting of hydrogen; hydroxy; sulfonate; halogen; alkoxy and cycloalkoxy groups comprising 1 to 6 carbon atoms, wherein said alkoxy and cycloalkoxy groups can be substituted with phenyl; and amine, wherein said amine can be substituted with alkyl, cycloalkyl, or phenyl.

BACKGROUND OF THE INVENTION

This application is a continuation-in-part of U.S. patent application Serial No. 042,383, filed April 23, 1987 entitled "N^6-Substituted 9-Methyladenines: A New Class of Adenosine Receptor Antagonists," which is incorporated herein by reference in its entirety.

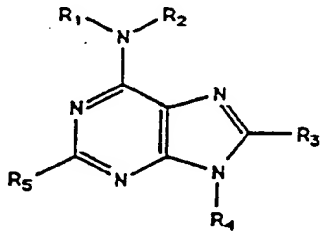
Adenosine receptors have been divided into two subtypes, based on adenylate cyclase activity: A_1 (R_i) receptors mediate inhibition and A_2 (R_a) receptors mediate stimulation of adenylate cyclase activity. Some N^6-substituted adenosine analogs, like N^6-R-phenyl isopropyl adenosine (R-PIA) have very high affinity for A_1 adenosine receptors, but at A_2 receptors 5'-N-ethylcarboxamido-adenosine (NECA) is more potent than N^6-substituted analogs. Alkylxanthines, such as caffeine and theophylline, are the best known antagonists at adenosine receptors.

Adenine was generally believed to have no effect on adenosine receptor-controlled systems. However, it was found that at low concentrations adenine displays specific competitive antagonism of adenosine-induced cyclic Amp accumulation in a human fibroblast cell line. Methylation of adenine at the 9-position increases potency about 4-fold

in this assay. At higher concentration, both compounds show non-specific inhibitory activity.

DETAILED DESCRIPTION OF THE INVENTION

The compounds of this invention are represented by the general formula:



wherein R_2 is selected from the group consisting of cycloalkyl radicals having from 3 to 8, preferably 3 to 7, ring carbon atoms, alkyl radicals having from 1 to 10 carbon atoms, aryl radicals having from 6 to 13, preferably 6 to 10, carbon atoms, aralkyl radicals having from 7 to 14, preferably 7 to 10, carbon atoms, and heteroatom- and halogen-substituted derivatives thereof wherein said heteroatom may be selected from the group consisting of nitrogen, phosphorus, sulfur and oxygen; R_1 may be hydrogen or R_2, and R_3 is selected from the group consisting of hydrogen, halogen, amine, carboxy, alkyl radicals having 1 to 10 carbon atoms, cycloalkyl radicals having from 3 to 8, preferably 5 to 6, ring carbon atoms, thio, sulfonate, sulfonamide, sulfon, sulfoxamide, phenyl, alkyl-substitued amine, and cycloalkyl substituted amine. R_4 is selected from the group consisting of benzyl, phenyl, and alkyl groups comprising from 1 to 4 carbon atoms, wherein said alkyl group can be substituted with oxygen, for instance ethers and alcohols. R_5 is selected from the group consisting of hydrogen; hydroxy; sulfonate; halogen; alkoxy and cycloalkoxy groups comprising 1 to 6 carbon atoms, wherein

said alkoxy and cycloalkoxy groups can be substituted with phenyl; and amine, wherein said amine can be substituted with phenyl and alkyl and cycloalkyl groups comprising 1 to 6 carbon atoms.

The preferred compounds are those wherein R_1 is hydrogen; wherein R_2 is endo-2-Norbornyl or cyclopentyl; wherein R_3 is bromine, chlorine, amino, hydrogen, thio, cyclopentyl or cyclopentylamine; wherein R_4 is methyl, ethyl, 2-hydroxy-ethyl, phenyl, or 2-hydroxyethoxy methyl; and wherein R_5 is hydrogen, hydroxy or chlorine.

The following is a list of compounds useful in the practice of the present invention. This list is intended to be illustrative and the scope of the invention is not limited to compounds named therein:

N^6-Cyclobutyl-9-Methyl Adenine (MA)
N^6-Cyclopentyl-9-MA
N^6-Methylcyclopentyl-9-MA
N^6-Cyclohexyl-9-MA
N^6-Methyl-9-MA
N^6-3-Pentyl-9-MA
N^6-Phenyl-9-MA
N^6-2-Fluorophenyl-9-MA
N^6-Benzyl-9-MA
N^6-2-Phenethyl-9-MA
N^6-2-(3,4,5-Trimethoxyphenyl)ethyl-9-MA
N^6-2-(3-Pyridylethyl)-9-MA
N^6-2-(3-Thienylethyl)-9-MA
N^6-R-1-Phenyl-2-propyl-9-MA
N^6-S-1-Phenyl-2-propyl-9-MA
N^6-(endo-2-Norbornyl)-9-MA
N^6-1-(2-Thienyl)-2-butyl-9-MA

N^6-(exo-2-Norbornyl)-9-MA
N^6-2,2-diphenylethyl-9-MA
N^6-2-phenylethyl-9-MA
N^6-2-(2-chlorophenyl)ethyl-9-MA
$N^6$1-indanyl-9-MA
N^6-2-aminoethyl-9-MA
N^6-(N,N-Dimethylaminoethyl)-9-MA
N^6-R-1-phenyl-1-ethyl-9-MA
N^6-S-1-phenyl-1-ethyl-9-MA
N^6-2-thienyl-9-MA
N6-(4-chloro-2-methyl phenyl)-9-MA
N^6- 2-(3-ethylindole)-9-MA
N^6-(1-methyl-2-phenylethyl)-9-MA
N^6-(1-methyl-2-phenoxyethyl)-9-MA
N^6-1-carboxy-1-butyl-9-MA
N^6-(endo-2-norbornyl)-2-chloro-9-MA
N^6-(endo-2-norbornyl)-8-cyclopentyl-9-MA
N^6-(endo-2-norbornyl)-8-hydroxy-9-MA
N^6-(endo-2-norbornyl)-8-bromo-9-MA
N^6-(endo-2-norbornyl)-8-amino-9-MA
N^6-(endo-2-norbornyl)-8-carboxy-9-MA
N^6-cyclopentyl-8-cyclopentyl-9-MA
N^6-(endo-2-norbornyl)-9-[(2-hydroxyethoxy)methyl]adenine
N^6-(endo-2-norbornyl)-8-thio-9-MA
N^6-(endo-2-norbornyl)-8-chloro-9-MA
N^6-(endo-2-norbornyl)-8-sulfonate-9-MA sodium salt
N^6-(endo-2-norbornyl)-2-hydroxy-9-MA
N^6-(endo-2-norbornyl)-8-cyclopentylamine-9-MA
N^6-(endo-2-norbornyl)-8-propylamine-9-MA
N^6-(endo-2-norbornyl)-9-phenyl adenine
N^6-cyclopentyl-2-chloro-9-MA
N^6-phenyl-2-chloro-9-MA
N^6-cyclopentyl-9-phenyl adenine
N^6-R-1-phenyl-2-propyl-9-phenyl adenine

N^6-S-1-phenyl-2-propyl-9-phenyl adenine
N^6-[(3-chloro-endo-2-norbornyl)]-9-MA
N^6-phenyl-9-phenyl adenine
2-ethoxy-9-MA
2-propoxy-9-MA
2-butoxy-9-MA
2-isopropoxy-9-MA
2-(2-butoxy)-9-MA
2-(2-methyl propoxy) 9-MA
2-pentoxy-9-MA
2-(2-phenylethoxy) 9-MA
2-phenylamino-9-MA
9-hydroxyethyladenine
N^6-cyclopentyl-9-benzyl adenine
N^6-cyclohexyl-9-ethyl adenine

The preparation of 9-methyl adenines is well known. See R. K. Robins, K. J. Dille, and B. E. Christensen, J. Org. Chem., 19, 930 (1954); R. K. Robins and H. H. Lin, J. Am. Chem. Soc., 79, 490 (1957; and J. A. Montgomery and Carroll Temple, Jr., J. Am. Chem. Soc., 79, 5238 (1957).

Preparation of N^6-Cyclopentyl-9-Methyl Adenine

To prepare N^6-cyclopentyl-9-methyl Adenine the following additional steps were taken. A mixture of 6-chloro-9-methyl Adenine (0.82g), cyclopentylamine (0.52 ml), triethylamine (0.53 ml) and ethanol (60 ml), was refluxed for 24 hours. The solution was concentrated in vacuo to a yellow syrup. The syrup was passed through a C-18 column to give 0.78g or 74% yield of with m.p. 108-109°C. ^{1}HNMR-(Me_2SO-d6): δ1-2(m,9 H); 3.7(S,CH_3); 7.6(d,NH); 8.1(S,1H); 8.2(S,1H).

Preparation of N^6-3-Pentyl-9-Methyladenine

A mixture of 6-chloro-9-methyladenine (1.5g), 3-pentylamine (1.3 ml), triethylamine (1.3ml) and ethanol (60 ml), was refluxed for 24 hours. The solution was concentrated and passed through a C-18 column to give a white solid having m.p. 107-109°C.

Preparation of N^6-(2-Aminonorbornyl)-9-methyl Adenine

A mixture of 1.5g 6-chloro-9-methyl Adenine, 1.75 g endo-2-aminonor-bornane, 2.9 ml triethylamine and 60 ml ethanol was refluxed overnight. The solution was then concentrated in vacuo and the remainder was passed through C-18 prep-chromatography to give 1.6g (75% yield) m.p. 130-131°C. ^{1}HNMR(Me_2SO-d6): δ1-2.6(m,10 H); 3.8(S, CH_3); 4.1(m,1H); 7.2(S,NH); 7.4(S,1H); 7.6(S,1H).

Preparation of N^6-(endo-2-Norbornyl)-8-Bromo-9-MA

To a stirred suspension of N^6-(endo-2-norbornyl)-9-MA (6g, 24.66 mmoles) in 150 ml of 1M sodium acetate buffer (pH 3.9) was added a solution of bromine (3.0 ml) in 300 ml of 1M sodium acetate buffer (pH 3.9). The mixture was stirred overnight and the resulting precipitate was filtered and washed with water. To the residue was added silica gel and the suspension was evaporated to a powder. The powder was added to a silica gel column (150g, packed with petroleum ether). The purine was eluted with 10% to 25% ethylacetate in petroleum ether. Evaporation of the appropriate fractions gave 6.7g, 84% yield of N^6-(endo-2-Norbornyl)-8-Bromo-9-MA.

Preparation of N^6-(endo-2-Norbornyl)-8-Azido-9-MA

To a solution of N^6-(endo-2-Norbornyl)-9-Bromo-9-MA (0.72g, 2.23 mmoles) in DMF was added sodium azide (0.91g, 13.98 mmoles). The mixture was heated at 70-80°C overnight. The crude was dissolved in water, extracted with ethyl

acetate, and then dried over magnesium sulfate and the organic phase was evaporated in vacuo to give 0.62g, 98% yield.

Preparation of N^6-(endo-2-Norbornyl)-8-Amino-9-MA

The crude product, N^6-(endo-2-Norbornyl)-8-Azido-9-MA (0.5g, 1.75 mmole) was dissolved in ethanol. The solution, in presence of 10% palladium on charcoal (1g), was shaken with H_2 at 35 atm overnight. The suspension was filtered and evaporated to a small volume, and then poured through a C-18 column (HPLC) to give 0.36g 80% yield of N^6-(endo-2-norbornyl)-8-Amino-9-MA.

Preparation of N^6-(endo-2-Norbornyl)-8-Oxo-9-MA

To a mixture of N^6(endo-2-Norbornyl)-9-Bromo-9-MA (0.15g, 0.62 mmole) in 12 ml acetic acid was added sodium acetate (0.5g) and 1.2 ml acetic anhydride. The mixture was allowed to reflux overnight. The mixture was then evaporated under vacuo and purified on a chromatotron using $CHCl_3$, stepping to 2% ethanol, and finally to 4% ethanol on 2 mm plate giving 90 mg, 75% yield of N^6-(endo-2-Norbornyl)-8-Oxo-9-MA.

Preparation of N^6-(endo-2-Norbornyl)-8-Cyclopentylamine-9-MA

To a solution of N^6-(endo-2-Norbornyl)-8-Bromo-9-MA (0.5g, 1.55 mmols) in 20 ml ethanol was added 20ml of cyclopentylamine; the reaction mixture was refluxed overnight. The mixture was then evaporated under vacuo and passed through a C-18 column (HPLC) to give 0.32g, 77% yield of N^6-(endo-2-Norbornyl)-8-Cyclopentylamine-9-MA.

Preparation of N^6-(endo-2-Norbornyl)8-Bromo-2-Chloro-9-MA

N^6(endo-2-Norbornyl)2-Chloropurine was first prepared as follows: A mixture of 2,6-dichloropurine (5.0g, 26.45 mmoles) endo-2-aminobornane hydrochloride (5.0g, 33.86

mmoles) and triethyl amine (10 ml) in absolute ethanol was refluxed for 48 hours. The solution was then cooled to room temperature and evaporated in vacuo to a white solid. The white solid was washed with water and dried to yield 6.0g, 84% yield of N^6-(endo-2-Norbornyl)2-Chloropurine used as is with no further purification for next step.

A mixture of N^6-(endo-2-Norbornyl)-2-chloropurine (5.0g, 18.96 mmoles), triethyl ammonium hydroxide (18.9 ml), and methyl iodide (1.41 ml, 22.68 mmoles) in dichloromethane was heated to 35°C for 24 hours. The solution was then evaporated in vacuo and the syrup was crystallized in methanol to give 4.0g, 76% yield of N^6-(endo-2-Norbornyl)2-chloro-9-MA.

To a stirred solution of N^6-(endo-2-Norbornyl)-2-chloro-9-MA (4.3g, 14.4 mmoles) in acetate buffer (1 molar acetic acid and 1 M sodium acetate mixture, 45:1 ratio respectively; pH ≡ 3.9) was added dropwise Bromine (3.12g, 19.56 mmoles) dissolved in the acetate buffer. The reaction mixture was stirred for 72 hours; the mixture was then filtered and the solid material collected was eluted from ethyl acetate/petroleum ether on silica gel column to yield 4.9g, 85% of N^6-(endo-2-Norbornyl)8-Bromo-2-Chloro-9-MA.

Preparation of N^6-(endo-2-Norbornyl)-8-Cyclopentyl-9-MA

To a vigorously stirred solution of 2g (12.2 mmoles) of 4-methylamino-5-amino-6-chloropyrimidine in $CHCl_3$ was added dropwise over a period of 20 minutes cyclopentane carbonyl chloride (1.6g, 12.2 mmoles). The mixture was stirred overnight and then evaporated in vacuo to a yellow syrup. The syrup was then dissolved in methanol and purified through a C-18 column (HPLC) to give 2.2g, 71% yield of 4 methylamino-6-chloro-5-cyclopentylamido-pyrimidine.

4-methylamino-6-chloro-5-cyclopentylamido-pyrimidine 2.2g, 8.6 mmoles) was refluxed in $POCl_3$ for approximately 2 hours. The solution was concentrated in vacuo to a syrup. The syrup was added dropwise to ice. The aqueous mixture was then extracted with chloroform. The organic layer was evaporated and the syrup was passed through a C-18 column (HPLC) giving 1.25g, 63% yield of 8-cyclopentyl-6-chloro-9-methyladenine.

A mixture of 8-cyclopentyl-6-chloro-9-methyladenine (0.48g 2.0 mmoles) and endo-2-aminonorbornane hydrochloride (0.5g, 3.4 mmoles) in absolute ethanol was refluxed for 48 hours. The mixture was then evaporated in vacuo and purified through a C-18 column (HPLC) to give 0.45g, 71% yield of N^6-(endo-2-Norbornyl)-8-cyclopentyl-9-MA.

Preparation of N^6-(endo-2-Norbornyl)-8-Chloro-9-MA

A mixture of N^6-(endo-2-Norbornyl)-8-bromo-9-MA (1.25g, 3.7 mmoles) and $POCl_3$ was refluxed for 1 hour. Then the phosphorous oxychloride was removed in vacuo and the yellow solid was passed through a C-18 column (HPLC) to give 0.96g, 84% yield of N^6-(endo-2-Norbornyl)-8-chloro-9-MA.

Preparation of N^6-(endo-2-Norbornyl)-9-[(2-hydroxyethoxy) methyl]purine.

To a solution of 6-chloropurine (6g, 38.8 mmoles) in DMF was added sodium hydride 60% (0.93g) over 1.5 hour period. (2-acetoxyethoxy)methyl bromide was then added at room temperature; the reaction mixture was allowed to stir for 2 hours under N_2 atmosphere. H_2O was added and the product was extracted with ethyl acetate. The organic phase was dried over MgSO4, filtered, and evaporated in vacuo to give a light yellow solid 7.1g, 68% yield of 9-[(2-Acetoxy-

ethoxy)methyl]-6-chloro-purine. The crude was used without further purification.

To a solution of 9-[(2-acetoxyethoxy)methyl]-6-chloro-purine (5.1g, 18.8 mmoles) in ethanol and triethylamine was added endo-2-aminonorbornane hydrochloride (4.0g, 27.1 mmoles). The mixture was refluxed in vacuo and the residue was purified by HPLC to give 4.70g, 77% yield of N^6-(endo-2-Norbornyl)-9-[(2-acetoxyethoxy)methyl]purine.

A solution of N^6-(endo-2-Norbornyl)-9-[(2-acetoxyethoxy) methyl]purine (3.75g, 10.8 mmoles) in methanol was saturated with NH_3 gas under N_2. The mixture was stirred overnight, then evaporated in vacuo to give 2.03g, 62% yield of N^6-(endo-2-Norbornyl)-9-[(2-hydroxyethoxy)methyl]purine.

The invention is further illustrated by the following examples which are illustrative of various aspects of the invention. These examples are not intended as limiting the scope of the invention as defined by the appended claims.

PHARMACOLOGIC TESTING

A series of N^6-substituted 9-methyladenines were assayed as adenosine antagonists in A_1 and A_2 test systems (Ukena, et al, FEBS Lett. 215(2), 203-208, 1987). For activity at A_1 receptors, compounds were tested as inhibitors of the binding of N^6-R-[^{3}H]-Phenylisopropyladenosine in rat brain membranes and for their ability to prevent R-PIA-induced inhibition of adenylate cyclase in rat fat cell membranes. For activity at A_2 receptors, compounds were tested as antagonists of NECA-stimulated adenylate cyclase in membranes of human platelets and rat PC12 cells.

It is known that A_1 receptors influence inhibition of adenylate cyclase in fat, brain and heart cells; whereas A_2 receptors stimulate adenylate cyclase in endothelial and smooth muscle cells. (See John W. Daly, et al., "Structure-Activity Relationship for N^6-Substituted Adenosines at a Brain A_1-Adenosine Receptor With A Comparison to an A_2-Adenosine Receptor Regulating Coronary Blood Flow," Biochemical Pharmacology, Vol. 35. No. 15, pp. 2467-2471 (1986)).

The results summarized below in Table I show that N^6 substitution can markedly increase the potency of 9-methyladenine at adenosine receptors. The lower apparent affinity values (K_B, K_i) identify the most potent compounds. The most pronounced effect is seen at A_1 receptors. For example, N^6-Cyclopentyl-9-methyladenine is at least 100-fold more potent than 9-methyladenine at A_1 receptors. At A_2 receptors, this compound is 5-fold more potent than 9-methyladenine in the human platelet assay. Thus, this data demonstrates the activity of a novel series of adenosine antagonists.

TABLE 1

	A_2 Effects: K_B (μM) vs NECA Stimulation (Adenylate Cyclase)		A_1 Effects: K_B (μM) vs PIA INHIBITION (Adenylate Cyclase)	A_1 Effects: K_i (μM) vs [^{3}H] PIA (Binding)
	(A)	(B)	(C)	(D)
1. Adenine	760	570	>1000	>100
2. 9-Methyladenine (9-MA)	24	24	112	106
N^6-substituted 9-methyladenines				
3. N^6-Cyclobutyl-0-MA	5.5	23	0.89	1.2
4. N^6-Cyclopentyl-9-MA	4.9	25	1.3	0.54
5. N^6-Methylcylopentyl-9-MA	45	56	9.0	2.5
6. N^6-Cyclohexyl-9-MA	7.4	21	0.65	0.94
7. N^6-Methyl-9-MA	150	130	220	>100
8. N^6-3-Pentyl-9-MA	11	53	7.6	3.3
9. N^6-Phenyl-9-MA	21	107	10	25
10. N^6-2-Fluorophenyl-9-MA	11	29	17	8.5
11. N^6-2-Benzyl-9-MA	57	100	49	17
12. N^6-2-Phenethyl-9-MA	170	120	>300	>100
13. N^6-2-(3,4,5-Trimethoxyphenylethyl)-9-MA	23	40	122	>100
14. N^6-2-(3-Pyridylethyl)-9-MA	92	117	96	41
15. N^6-2-(3-Thienylethyl)-9-MA	14	25	24	20
16. N^6-R-1-Phenyl-2-propyl-9-MA	13	25	7.2	2.5
17. N^6-S-1-Phenyl-2-propyl-9-MA	23	74	23	10

(A) - Human Platelet Membranes

(B) - Rat PC12 Membranes

(C) - Rat Fat Cell Membranes

(D) - Rat Brain Membranes

FURTHER FUNCTIONAL ASSAYS

To test the selectivity of the compounds of the invention, in vitro assays were conducted utilizing model tissues that are thought to contain homogenous populations of either the A_1 or A_2 adenosine receptors. Four examples were characterized by their ability to antagonize competitively the action of adenosine agonists in eliciting two responses: the reduction in force of contraction of guinea pig atrium (A_1); and the decrease in the contractile tone of the guinea pig taenia caecum (A_2).

The left atria from male guinea pigs were isolated, suspended between two punctate electrodes, and placed in a 20 ml organ bath that contained Krebs-Hensileit solution that was continuously gassed with 95% O_2 + 5% CO_2 and maintained at 31°C. The resting tension was one gram. The atria were stimulated electrically at 1 Hz, 1 msec duration pulses at supramaximal voltage. The force of contraction was recorded isometrically.

Taenia from the guinea pig caecum were cut into lengths of 1.5-2 cm. The tissues were suspended in a 20 ml organ bath containing de Jalon's solution that was gassed with 95% O_2 + 5% CO_2 and maintained at 31°C. The resting tension was 1.5 g. The contractile response was measured isotonically. Tissues were contracted with 10^{-7}M 5-methyl-furmethide and allowed sufficient time to reach a stable contraction before addition of adenosine agonists.

The ability of the compounds to antagonize the effects of agonists was analyzed using modified Schild plots.

Although there was some sensitization of the tissue, i.e. addition of the agonist produced a larger response in the presence of high concentrations of the subject compounds, N^6-3-Pentyl-9-MA, N^6-Cyclopentyl-9-MA and N^6-(endo-2-Norbornyl)-9-MA did not competitively antagonize the effects of adenosine agonists in relaxing the taenia caecum. Sensitization is also observed when using high concentrations of 8-phenyltheophylline (8-PT), a non-selective adenosine receptor antagonist. 8-PT did antagonize the effects of agonists at low concentrations. The lack of competitive antagonism by the other compounds suggests that the latter compounds do not interact appreciably with A_2-adenosine receptors and are, thus, selective for A_1 adenosine receptors.

However, N^6-3-Pentyl-9-MA, N^6-Cyclopentyl-9-MA, N^6-(endo-2-Norbornyl)-9-MA and N^6-4-(2-thienyl)-3-butyl)-9-MA all were found to be competitive antagonists at adenosine receptors in the atria. N^6-3-Pentyl-9-MA and N^6-1-(2-thienyl)-2-butyl-9-MA also produced increases in basal force of contraction in the atria. Affinity constants (pK_B) for the present compounds determined using known methods are summarized in Table 2 below:

Table 2

Drug	pK_B
N^6-3-Pentyl-9-MA	5.4 ± 0.14
N^6-Cyclopentyl-9-MA	6.17 ± 0.11
N^6-(endo-2-Norbornyl)-9-MA	6.28 ± 0.09
N^6-1-(2-Thienyl)-2-butyl)-9-MA	5.36 ± 0.1

These results show that the above examples display selectivity towards the A_1 adenosine receptor, with N^6-(endo-2-Norbornyl)-9-MA being the most potent antagonist.

IN VIVO ASSAY

In vitro selectivity of the present antagonists was confirmed by in vivo tests on rat heart rate and blood pressure, the former associated with A_1 receptors and the latter associated with A_2 receptors.

Rats were anesthetized with urethan and blood pressure was monitored via a carotid cannula. Drug injections were made intravenously through a jugular cannula. Blood pressure, EGC, and heart rate were recorded on a Grass polygraph.

Adenosine produced a dose dependent decrease in blood pressure and heart rate, with a concommitant increase in the P-R interval of the ECG. Administration of N^6-(endo Norbornyl)-9-methyladenine attenuated the effects of subsequently administered adenosine on all parameters measured. At high doses, adenosine causes heart block; this effect was also substantially reduced by the agonist. Due to the short duration of action and direct route of administration of adenosine, it is often difficult to determine whether adenosine decreased blood pressure by causing peripheral vasodilation or by reducing cardiac output. To overcome these problems, NECA (5'-N-ethylcarboxamide adenosine), which is longer-acting and selective for A_2 adenosine receptors, was used as an adenosine receptor agonist. Prior administration of N-0861 attenuated the effects of NECA on the heart while minimally affecting the NECA-induced decrease in blood pressure. These results show that N^6-endo-2-Norbornyl)-9-methyladenine is a cardioselective adenosine receptor antagonist in vivo and support the data above showing selectively of the N-6 substituted 9-methyladenines of the invention as A_1 adenosine receptor antagonists.

FURTHER RECEPTOR AFFINITY ASSAYS

Further tests to discover the affinities of test compounds at A_2 receptors were conducted. [^{3}H]-N-ethylcarboxamido adenosine ([^{3}H-]-NECA) was used as the radioligand, bovine caudate was the source of membranes, and the assay buffer was 50 mM Tris; 10 mM $MgCl_2$, pH 7.4.

To provide bovine caudate nuclei, bovine brains were obtained fresh from a local slaughterhouse. The caudate nuclei were dissected out and homogenized in Buffer A (50 mm Tris; 1 mm Na_2-EDTA; 5 mm KCl; 1 mm $MgCl_2$; 2 mm $CaCl_2$; pH 7.4) using a Brinkman Polytron. The homogenate was centrifuged at 40,000 x g for 20 minutes and washed once. The pellet was resuspended in Buffer A, incubated at 37°C for 15 minutes, then centrifuged. The pellet was washed once more, resuspended to a protein concentration of 5-10 mg/ml in Buffer A and frozen at -70°C until use.

The A_2 assays also contained 50 nM cyclopentyladenosine to block the binding of [^{3}H]-NECA to A_1 receptors (Bruns et al, 1986) and 1 unit/ml adenosine deaminase to degrade endognous adenosines. Varying concentrations of test compounds were incubated with the appropriate radioligand and membrane source for 1 hr at room temperature.

Assays were terminated by filtration over Whatman GF/B filters that had been pre-soaked with 0.1% polyethyleneimine using a 24 port Brandell cell harvester. The filters were washed three times with 3 ml of ice cold buffer and transfered to plastic scintillation vials to which 4 ml of Beckman Ready-Protein scintillation cocktail was added. The tubes were shaken and counted in a Beckman 3801 scintillation counter that converted cpm to dpm.

Data were analyzed by utilizing the Ligand® commercial computer program (Munson and Rodbard, 1980).

The results of these tests, expressed as the molar concentration of test compound needed to displace 50 percent of the [^{3}H]-CHA radioligand from rat cortical A_1 receptors, are summarized in Table 3 below:

Table 3

Adenosine Antagonists

Sample No.	Name	Rat Cortical Binding Constant Ki (M)
0861	N^6-(endo-2-norbornyl)-9-MA	11.6×10^{-8}
0913	N^6-(endo-2-norbornyl)-2-chloro-9-MA	10.5×10^{-8}
0966	N^6-2,2-diphenylethyl-9-MA	$>10^{-5}$
0967	N^6-2(2-chlorophenylethyl)9-MA	$>10^{-5}$
0982	N^6-2-Aminoethyl-9-MA	$>10^{-5}$
0983	N^6-(2,2-N-dimethylethyl)-9-MA	$>10^{-5}$
0840	N^6-cyclopentyl-9-MA	37.5×10^{-8}
0984	N^6-R-1-phenyl-1-ethyl-9-MA	$>10^{-5}$
0985	N^6-S-1-phenyl-1-ethyl-9-MA	$>10^{-4}$
0986	N^6-S-1-phenyl-2-propyl-9-MA	$>10^{-5}$
0987	N6 2-thienyl-9-MA	$>10^{-4}$
0988	N6(4-chloro-2-methylphenyl)-9-MA	$>10^{-5}$
0989	N^6-2-(3-ethylindole)-9-MA	$>10^{-5}$
0990	N^6-2-(phenethyl)9-MA	$>10^{-5}$
1001	N^6-(endo-2-norbornyl)-8-oxo-9-MA	$\approx 10^{-5}$
1002	N^6-2-(3,4,5-trimethoxyphenyl)ethyl-9-MA	$>10^{-5}$
1003	N^6-(endo-2-norbornyl)-8-bromo-9-MA	1.3×10^{-8}
1004	N^6-1-carboxy-1-butyl-9-MA	$>10^{-4}$
1005	N^6-(endo-2-norbornyl)-8-amino-9-MA	87×10^{-8}
1006	N^6-(endo-2-norbornyl)-8-carboxy-9-MA Sodium Salt	$>10^{-5}$

1059	N^6-(endo-2-norbornyl)9-[(2 hydroxyethoxy) methyl]adenine	49×10^{-8}
1060	N^6-(endo-2-norbornyl)-8-thio-9-MA	37×10^{-8}
1061	N^6-(endo-2-norbornyl)-8-chloro-9-MA	1.5×10^{-8}
1062	N^6-(endo-2-norbornyl)-8-sulfonate-9-MA Sodium Salt	$>10^{-4}$
1063	N^6-(Endo-2-norbornyl)-2-oxo-9-MA	112×10^{-8}
1064	N^6-(endo-2-norbornyl)-8-cyclopentyl-amine-9-MA	190×10^{-8}
0964	N^6-(endo-2-norbornyl)-8-cyclopentyl-9-MA	24×10^{-8}
0965	N^6-cyclopentyl-8-cyclopentyl-9-MA	14.1×10^{-8}
0978	N^6-(exo-2-norbornyl)-9-MA	43×10^{-8}

The compounds in Table 3 for which a solution having a concentration greater than 10^{-5}M was required to displace 50 percent of the radioligand are deemed ineffective as A_1 adenosine receptor antagonists.

In further experiments designed to determine the selectivity of N^6-endo-2-Norbornyl-9-methyl adenine at A_1 receptors, [^{3}H]-cyclohexyladenosine ([^{3}H]-CHA) was used as the radioligand, rat cortical membranes were the receptor source, and the assay buffer was 50 mM Tris; 2 mM $MgCl_2$ pH 7.4.

Male Sprague Dawley rats were killed by decapitation and the brains removed. The cerebral cortices were homogenized in 50 mm Tris; 2mm $MgCl_2$ (pH 7.4), and centrifuged at 40,000 x g for 10 minutes. The pellet was washed once, resuspended in Tris/$MgCl_2$ and incubated with 8 units/ml adenosine deaminase at 37°C for 30 minutes. The homogenate was centrifuged, washed once, resuspended to a protein concentration of 5-10 mg/ml and frozen at -70°C until use. The results in Table 4 below show that the test compound has

170 times more affinity for A_1 receptors than for A_2 receptors.

Table 4

Selectivity of N^6-endo-2-Norbornyl-9-MA
Bovine Caudate Binding Constants

At A_1 Receptors	At A_2 Receptors
K_i (M)	K_i (M)
4.1×10^{-8}M	6.96×10^{-6}M
	$A_1/A_2 = 5.89 \times 10^{-3}$
	= 170 fold selective for A_1 receptors

References

Munson, Peter J. and Rodbard, David (1980). "Ligand: A Versatile Computerized Approach for Characterizing Ligand-Binding Systems." Anal. Biochem. 107:220-239.

Bruns, Robert F., Lee, Gina H., and Pugsley, Thomas A. (1986) "Characterization of the A_2 Adenosine Receptor Labeled by ^{3}H-NeCA in Rat Striatal Membranes," Mol. Pharmacol. 29:331-346.

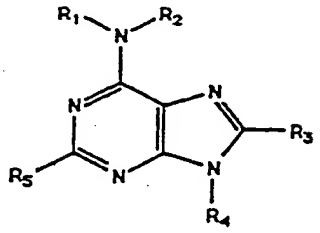
These N^6-substituted adenines are antagonists of A_2-adenosine receptor-mediated stimulation of adenylate cyclase in A_2-adenosine receptors and antagonists of A_1-adenosine receptor-mediated inhibition of adenylate cyclase. These compounds are useful in reversal of adenosine-mediated lipolysis, reversal of adenosine-mediated deleterious cardiovascular effects (conduction defects, hypotension), reversal of adenosine-mediated vascular actions in kidney, bronchodilation, antiarrhythmic action, reversal of adenosine-mediated relaxation of smooth muscle, anti-narcoleptic

action, CNS stimulation, and blockade of adenosine mediated inhibition of neurotransmitter release.

While particular embodiments of the invention have been described it will be understood of course that the invention is not limited thereto since many obvious modifications can be made and it is intended to include within this invention any such modifications as will fall within the scope of the appended claims.

What is claimed is:

1. Novel compounds represented by the general formula:



wherein R_2 is selected from the group consisting of cycloalkyl radicals having from 3 to 8 ring carbon atoms, alkyl radicals having from 1 to 10 carbon atoms, aryl radicals having from 6 to 13 carbon atoms, aralkyl radicals having from 7 to 14 carbon atoms and halogen- and heteroatom-substituted derivatives thereof wherein said heteroatom may be selected from the group consisting of halogen, nitrogen, phosphorus, sulfur and oxygen; R_1 may be hydrogen or R_2, and R_3 is selected from the group consisting of hydrogen, halogen, amine, carboxy, thio, sufonate, sulfonamide, sulfone, sulfoxamide, phenyl, alkyl- or cycloalkyl-substituted amine, alkyl radicals having 1 to 10 carbon atoms and cycloalkyl radicals having from 3 to 8 ring carbon atoms. R_4 is selected from the group consisting of benzyl, phenyl, and alkyl groups comprising from 1 to 4 carbon atoms wherein said alkyl group can be substituted with oxygen; and R_5 is selected from the group consisting of hydrogen, hydroxy, halogen, alkoxy and cycloalkoxy groups comprising 1 to 6 carbon atoms, wherein said alkoxy and cycloalkoxy groups can be substituted with phenyl; and amine wherein said amine can be substituted with members of the group consisting of phenyl, and alkyl and cycloalkyl, having 1 to 6 carbon atoms.

2. The compound of claim 1 wherein R_4 is methyl.

3. The compound of claim 2 wherein R_1 is hydrogen.

4. The compound of claim 3 wherein R_2 is a cycloalkyl having from 4 to 8 carbon atoms in the ring.

5. The compound of claim 3 wherein R_2 is phenyl or a substituted phenyl.

6. The compound of claim 3 wherein R_2 is 2-norbornyl, cyclopentyl and R_3 is selected from the group consisting of hydrogen, cyclopentyl, oxo, bromo, amino, carboxy, thio, chloro, fluoro, sulfonate, sulfonamido, cyclopentylamino, cyclopentyl, and physiologically acceptable salts thereof.

7. The compound of claim 3 wherein R_2 is selected from the group consisting of benzyl, phenyl, o-fluorophenyl, 3,4,5-trimethoxyphenylethyl, 3-pentyl, 2-phenylethyl, 2-(2-chlorophenylethyl); 1-indanyl, 2-aminoethyl, N,N-dimethyl-aminoethyl, 2-thienylbutyl, and cyclohexyl.

8. The compound of claim 3 wherein R_2 is endo-2-Norbornyl and R_4 is phenyl or (2-hydroxyethoxy)methyl.

9. The compound of claim 1 selected from the group consisting of N^6-(endo-2-Norbornyl)-9-[(2-hydroxyethoxy) methyl]adenine, N^6-(endo-2-Norbornyl)-8-thio-9-methyl adenine, N^6-(endo-2-Norbornyl)-8-chloro-9-methyl adenine, N^6-(endo-2-Norbornyl)-2-oxo-9-methyl adenine, N^6-(endo-2-Norbornyl)-8-cyclopentylamino-9-methyl adenine, N^6-cyclopentyl-9-methyl adenine, N^6-(endo-2-Norbornyl)-9-methyl adenine, N^6-(endo-2-norbornyl)-8-bromo-9-MA, N^6-cyclopentyl-8-cyclopentyl-9-methyl adenine, N^6-(exo-2-norbornyl)-9-MA, N^6-cyclopentyl-2-chloro-9-methyl adenine, N^6-[(3-chloro)-endo-2-norbornyl]-9-MA, N^6-cyclopentyl-9-phenyl adenine, N^6-

(endo-2-norbornyl)-8-cyclopentyl-9-MA, N^6-cyclopentyl-9-benzyl adenine, and N^6-(endo-2-norbornyl)-8-amino-9-MA.

10. The compound of claim 2 wherein R_5 is selected from the group consisting of hydrogen, ethoxy, methoxy, propoxy, n-butoxy, isopropoxy, 1-methylpropoxy, 2-methylpropoxy, 2-phenyl-ethoxy, methylamino, butylamino and anilino.

11. The compound of claim 3 wherein R_5 is chloro.

12. The compound of claim 3 wherein R_2 is selected from the group consisting of 3-pentyl, 1-phenyl-2-propyl, and phenyl.

13. The compound of claim 2 wherein R_2 is hydrogen and R_5 is selected from the group consisting of ethoxy, methoxy, propoxy, n-butoxy, isopropoxy, butyl-2-oxy, 2-methylpropoxy, pentoxy, 2-phenylethoxy, methylamino, butylamino and anilino.

14. The compound of claim 11 wherein R_2 is 2-(3-thienylethyl).

15. The compound of claim 7 wherein R_2 is cyclohexyl.

16. The compound of claim 11 wherein R_2 is 2-(3-pyridylethyl).

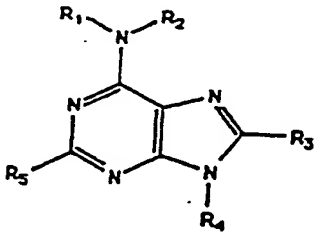
17. The method of antagonizing the A_2-adenosine receptor-mediated stimulation of adenylate cyclase which comprises administering to a subject an effective amount of one or more of the compounds of claim 4, 6, 7, 8 or 9.

18. The method of claim 17 wherein said subject is a human.

19. The method of antagonizing the A_1-adenosine receptor-mediated inhibition of adenylate cyclase which comprises administering to a subject an effective amount of a compound of claim 4, 6, 7, 8 or 9.

20. The method of claim 19 wherein said subject is a human.

21. The method of antagonizing the adenosine receptor which comprises administering to a subject an effective amount of a compound selected from the group of compounds represented by the general formula

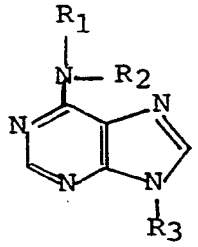


wherein R_2 is selected from the group consisting of cycloalkyl radicals having from 3 to 8 ring carbon atoms, alkyl radicals having from 1 to 10 carbon atoms, aryl radicals having from 6 to 13 carbon atoms, aralkyl radicals having from 7 to 14 carbon atoms, and halogen- and heteroatom-substituted derivatives thereof wherein said heteroatom may be selected from the group consisting of halogen, nitrogen, phosphorus, sulfur and oxygen; R_1 may be hydrogen or R_2, and R_3 is selected from the group consisting of hydrogen, halogen, amine, carboxy, thio, sufonate, sulfonamide, sulfone, sulfoxamide phenyl, alkyl- or cycloalkyl-substituted amine, alkyl radicals having 1 to 10

carbon atoms and cycloalkyl radicals having from 3 to 8 ring carbon atoms. R_4 is selected from the group consisting of benzyl, phenyl, and alkyl groups comprising from 1 to 4 carbon atoms wherein said alkyl group can be substituted with oxygen; and R_5 is selected from the group consisting of hydrogen, hydroxy, amine, halogen, alkoxy and cycloakoxy groups comprising 1 to 6 carbon atoms, wherein said alkoxy and cycloalkoxy groups can be substituted with phenyl; and amine, wherein said amine can be substituted with members of the group consisting of phenyl, alkyl, cycloalkyl, having 1 to 6 carbon atoms.

22. The method of claim 21 wherein said subject is a human.

23. Novel compounds represented by the general formula:



wherein R_1 is selected from the group consisting of cycloalkyl radicals having from 3 to 7 ring carbon atoms, alkyl radicals having from 2 to 10 carbon atoms, aryl radicals having from 6 to 10 carbon atoms, aralkyl radicals having from 7 to 10 carbon atoms and heteroatom substituted derivatives thereof wherein said heteroatom may be selected from the group consisting of halogen, nitrogen, phosphorus, sulfur and oxygen; R_2 may be hydrogen or R_1, and R_3 is an alkyl group comprising from 1 to 4 carbon atoms.

24. The compound of claim 23 wherein R_3 is methyl.

25. The compound of claim 24 wherein R_2 is hydrogen.

26. The compound of claim 25 wherein R_1 is a cycloalkyl having from 4 to 6 carbon atoms in the ring.

27. The compound of claim 25 wherein R_1 is phenyl or a substituted phenyl.

28. The compound of claim 27 wherein R_1 is selected from the group consisting of phenyl, o-fluorophenyl and 3,4,5-trimethoxyphenyl.

29. The compound of claim 25 wherein R_1 is benzyl or 2-phenylethyl.

30. The compound of claim 25 wherein R_1 is 2-(3-pyridylethyl) or 2-(3-thienylethyl).

31. The compound of claim 25 wherein R_1 is 3-pentyl.

32. The compound of claim 24 wherein R_2 is selected from the group consisting of methyl and 2-propyl and R_1 is selected from the group consisting of cyclopentyl and phenyl.

33. The compound of claim 30 wherein R_1 is 2-(3-thienylethyl).

34. The compound of claim 32 wherein R_2 is 2-propyl and R_1 is phenyl.

35. The compound of claim 28 wherein R_1 is benzyl.

36. The compound of claim 30 wherein R_1 is 2-(3-pyridylethyl).

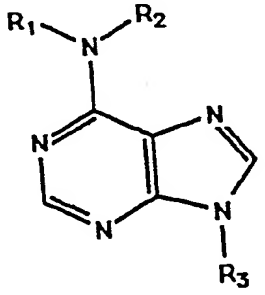
37. The method of antagonizing the A_2-adenosine receptor-mediated stimulation of adenylate cyclase which comprises administering to a subject an effective amount of one or more of the compounds of claims 26, 28, 31, 33 or 34.

38. The method of claim 37 wherein said subject is a human.

39. The method of antagonizing the A_1-adenosine receptor-mediated inhibition of adenylate cyclase which comprises administering to a subject an effective amount of a compound of claim 26, 28, 31, 33, 34, 35 or 36.

40. The method of claim 39 wherein said subject is a human.

41. The method of antagonizing the adenosine receptor which comprises administering to a subject an effective amount of a compound selected from the group of compounds represented by the general formula



wherein R_1 is selected from the group consisting of cycloalkyl radicals having from 3 to 7 ring carbon atoms, alkyl radicals having from 1 to 10 carbon atoms, aryl

radicals having from 6 to 10 carbon atoms, aralkyl radicals having from 7 to 10 carbon atoms and heteroatom substituted derivatives thereof wherein said heteroatom may be selected from the group consisting of halogen, nitrogen, phosphorus, sulfur and oxygen; R_2 may be hydrogen or R, and R_3 is an alkyl group comprising from 1 to 4 carbon atoms.

42. The method of claim 41 wherein said subject is a human.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/00210

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 31/52, C07D 473/34 C07D 473/18

U.S. CL.: 514/261,514/266, 544/277, 544/276,514/262

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S. CL. IPC(5)	514/261, 514/266, 544/277, 544/276, 514/262 A61K 31/52

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	FEBS Letters, vol.215, no.2, issued May 1987, (Ukena, et al.), "N^6-substituted 9-methyladenines: A New Class of Adenosine Receptor Antagonists.", pages 203-208. All pages.	1-42
X	GB, A, 953,897, Shell International Research Maatschappij N.V., 02 April 1964, see pages 1,3.	1-16,23-36
X	CA, A, 657,337, (Zwahlen), 05 February 1963.	1-16,23-36
X	JA, B2, 45,758, (Fujisawa Pharmaceutical Co. Ltd.), 17 November 1972.	1-16,23-36
X	GB, A, 2,041,359 (Freeman, et al.) 10 September 1980.	1-16,23-36

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

29 MARCH 1990

Date of Mailing of this International Search Report

05 JUL 1990

International Searching Authority

ISA/US

Signature of Authorized Officer

for DIANA G. RIVERS

Ngoc Ho Nguyen

HO NGUYEN

FURTHER INFORMATION C NTINUED FROM THE SEC ND SHEET

X	Journal of the American Chemical Society, vol. 79, no. 2, issued 20 January 1957, (Robins, et al.), "Potential Purine Antagonists. IV. Synthesis of Some 9-Methyl-6-substituted-purines", pages 490-494, see pages 490-494.	1-16,23-36
X	Journal of the American Chemical Society, vol. 79, no. 19, issued 05 October 1957, (Montgomery, et al.), "Synthesis of Potential Anticancer Agents. IX. 9-Ethyl-6-substituted-purines", pages 5238-5242. See all pages.	1-16,23-36

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers..... . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

CITATION OF DOCUMENT (Continued)

X	Journal of Organic Chemistry, vol. 28, no. 8, issued August 1963, (Myers, et al.). "Alkylation of the Purine Nucleus by Means of Quaternary Ammonium Compounds. I. Tetraalkylammonium Hydroxides", pages 2087-2089.	1-16,23-36
X	Chemical Abstracts, vol. 53, no. 15, issued 10 August 1959, (H.E. Skipper, et al.), "Structure-activity relations and cross-resistance observed on evaluation of a series of purine analogs against experimental neoplasms", abstract no. 14344i-14345b, Cancer Research 19, 425-37 (1959).	1-16,23-36
X	Chemical Abstracts, vol. 56, no. 9, issued 30 April 1962, (Ernst Carstens, et al.), "6,9-Disubstituted purine derivatives", abstract no. 10167i-10168d, Ger. (East) 21,223 (Cl. 12p.) Appl. May 29, 1958.	1-16,23-36
X	Chemical Abstracts, vol. 68, no. 23, issued 03 June 1968, (O.N. Kulaeva, et al.), "The effect produced by variations in the structure of cytokinins on their physiological activity", abstract no. 104032g, Dokl. Akad. Nauk, SSSR 178(5), 1204-(1968) (Russ).	1-16,23-36
X	Chemical Abstracts, vol. 70, no. 1, issued o6 Jan. 1969, (M.F. Petrova, et al.), Synthesis of potentially antiblastic substances related to 5-hydroxytryptamine (serotonin), abstract no. 4056r.	1-16,23-36
X	Chemical Abstracts, vol. 78, no. 13, issued 02 April 1973, (Kamiya, et al.), "4-(9H-Purin-6-yl) amino-4-deoxy-D-erthyronate esters, Abstract no. 84764y, Japan 72 45,757(Cl.Co7d,A61K), 17 Nov. 1972, Appl. 70 26092, 28 Mar. 1979; 3pp.	1-16,23-36

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Chemical Abstracts, vol. 79, no. 19, issued 12 Nov. 1973, (J. E. Fox et al.), "Effect of substituents at the 9-position on cytokinin activity", abstract no. 112299s, Phytochemistry 1973, 12(7), 1531-3 (Eng.).	1-16,23-36
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